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**14. ABSTRACT**

Elucidating and modeling irradiation effects on centrosomal and chromosomal stability within breast cancer.
Background: At the cellular level, ionizing radiation (IR) represents an empirical and reproducible insult that elicits a well-characterized cellular response. Genetic alterations, cell cycle effects and IR-induced chromosomal instability are defined-by-products of irradiation as is centrosomal amplification. The centrosome represents the major microtubule organizing center of the dividing cell and along with the nucleus, is precisely replicated during each cell cycle. It is postulated that centrosomal amplification translates into tetraploid, through mitotic catastrophe, or aneuploid, through aberrant division, daughter cells. At this tissue level, centrosomal deregulation has been identified within the majority of malignancies and is positively correlated with chromosomal instability, higher grade tumors and patient survival. At the cellular level, we would like to investigate the mitotic outcomes downstream of irradiation induced centrosomal amplification and develop a mathematical model for this process that can be translated to different genetic backgrounds and, in the future, different micro environmental cues and tissues.

**15. SUBJECT TERMS**
breast cancer, centrosomal and chromosomal stability

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**Introduction**

Aneuploidy, or abnormal genetic content, characterizes many tumors, particularly those of epithelial origin. Current literature cites chromosomal instability (CIN) as a primary cause of aneuploidy and particularly pathogenic consequence of cancer. Aneuploidy and CIN have been identified within early breast tumors, linked to cancer grade and progression, and shown to be predictive of patient response to therapy and outcome. It is speculated that aneuploidy and CIN create genomic variation leading to an evolutionary selection of malignant cells. Like the karyotype, the centrosome represents an important marker within breast tumors. While a causative relationship between centrosomal abnormalities (CA), aneuploidy and CIN is assumed through correlative studies, there is a surprising dearth of direct examination of causality between these central indicators of cancer diagnosis, development and therapeutic response. The primary goal of this application was to characterize, quantify and model the earliest stages of genomic instability by relating the frequency of CA, aneuploidy and CIN induced by exogenous and endogenous perturbation of non-malignant human mammary epithelial cells (HMEC).

**Body**

The DOD BCRP BC050612 funded research conducted at LBNL, under the supervision of Christopher Maxwell Ph.D., from July 2006 through January 2007. Dr. Maxwell received a job offer at the Catalan Institute for Oncology starting March 2007. LBNL did not release the remaining funds for the DOD BCRP BC050612 and so the project terminated February 01, 2007.

Progress on BC050612 was advancing as outlined in the SOW. Dr. Maxwell was a secondary author on two publications outlining software for the high throughput quantitation of centrosome amplification within murine tissues and mammalian cell lines (see below). Moreover, Dr. Maxwell is primary author on a report outlining a role for TGF-beta in the supervision of spontaneous centrosome amplification in vitro and in situ (see below, submitted to Cancer Research Jan '07). Life cell microscopic capabilities are being established at LBNL and an MCF7-hCnt2:GFP cell line is available for control experiments following centrosome amplification in real time.

**Key Research Accomplishments/Reportable outcomes**

Research outcomes have been described in the following publications:

Raman S, Maxwell CA, Barcellos-Hoff MH, Parvin B. Geometric approach to segmentation and protein localization in cell culture assays. Journal of Microscopy. 2007 Jan; 225 (Pt. 1): 22-30.

Fleisch MC, Maxwell CA, Kuper CK, Brown ET, Barcellos-Hoff MH, Costes SV. Intensity based signal separation algorithm for accurate quantification of clustered centrosomes in tissue sections. Microscopy Research and Techniques. 2006 Dec; 69(12): 964-72.

Additionally, the following manuscript was submitted to Cancer Research:

Maxwell CA, Fleisch , MC, Boissière A, Erickson A, Costes SV, Parvin B, Barcellos-Hoff MH. TGFβ prevents genomic instability by selectively deleting epithelial cells with centrosome amplification. Cancer Research (submitted Jan' 07)

The abstract for the preceding manuscript is as follows:

Genetic polymorphisms that compromise signaling by transforming growth factor β1 (TGFβ) are associated with increased breast cancer risk. Since TGFβ signaling is required for the immediate and early responses to DNA damage (Cancer Res **66**:10861-68, 2006), chronically reduced signaling could augment genomic instability and, hence, neoplastic risk. To test this prediction, we measured centrosome amplification (CA), which is an early event in breast cancer that promotes tetraploidy and genomic instability, in *Tgfβ1* heterozygote

mammary epithelium. CA was significantly increased compared to wildtype at 6 month of age and further increased in 18 month old mice. We then investigated the genomic stability of non-malignant human mammary epithelial cells cultured under conditions that mimic the low TGFβ signaling genotype. TGFβ inhibition significantly increased CA frequency, tetraploidy and aneuploidy. Addition of TGFβ suppressed all three measures of genomic instability while increasing apoptosis, suggesting that aberrant cells were selectively deleted. Genomic instability induced by TGFβ inhibition was overcome by p53 activation using an mdm-2 inhibitor, consistent with p53-mediated apoptosis. Mammary reconstitution experiments demonstrate genetic epistasis between TGFβ and p53 pathways in control of CA. TGFβ inhibition reduced p53 localization to mitotic centrosomes, which depends on ataxia telangiectasia mutated (ATM) kinase activity. Caffeine, which inhibits ATM, blocked TGFβ deletion of cells with CA. These data suggest that a novel tumor suppressor function of TGFβ acts via p53 and ATM to selectively delete genomically unstable epithelial cells.